

REMARKS

Initially, Applicants thank Examiners Mahatan and Allen for the courtesies extended in the interview of July 16, 2003.

Claims 5 and 35-40 have been canceled without prejudice or disclaimer. Claims 41-57 have been added and therefore are pending in the present application.

Claims 41-47 are supported by the specification and claims as originally filed. Claims 41-47 correspond to the subject matter of cancelled claims 5 and 35-40. New claim 41 recites "wherein said parent maltogenic alpha-amylase has an active site which comprises a cluster of three amino acid residues corresponding to positions D329, D228 and E256 in SEQ ID NO:2." Support is found in original claim 5 and in the specification at page 5, lines 21-23.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Rejection of Claims 5 and 35-40 under 35 U.S.C. 112, Enablement

Claims 5 and 35-40 are rejected under 35 U.S.C. 112, first paragraph, as allegedly lacking enablement. The Examiner alleges that Applicants:

(1) fail to sufficiently describe which active site residues are identified based on electrostatic or hydrophobic interactions and are thereby changed;

(2) fail to identify the residues which are within 15 Å from the active site and are involved in electrostatic or hydrophobic interactions with an active site residue; and

(3) fail to indicate the amino acid residue which when utilized to substitute with the amino acid residue of the parent enzyme would change the electrostatic and/or hydrophobic surroundings of an active site, and which can be accommodated in the structure.

As applied to the new claims, this rejection is respectfully traversed.

The claims are directed to a method for constructing a variant of a parent maltogenic alpha-amylase. The parent maltogenic alpha-amylase is defined in the claims as having "an amino acid sequence which is at least 70% identical to amino acid residues 1-686 of SEQ ID NO:2." By virtue of its identity to SEQ ID NO:2, the recited parent maltogenic-alpha amylases will accordingly have amino acid sequence, including its active site amino acid residues, which are generally identical to the amino acids in SEQ ID NO:2 or which correspond to amino acids in SEQ ID NO:2. See the specification, e.g., at page 7, lines 23-36. The determination of what is a corresponding amino acid is a task that is well within the skill of the artisan and can be readily

determined between highly related proteins (including those which have 70% identity to each other), e.g., by aligning the parent maltogenic-alpha amylase to the reference sequence. See the specification at page 20, lines 10-21.

The claims further recite the step of preparing a three dimensional structure of the parent maltogenic alpha amylase, namely by "modeling the parent alpha-amylase on the three-dimensional structure of amino acid residues 1-686 of SEQ ID NO:2 depicted in the Appendix to produce a three-dimensional structure of the parent alpha-amylase." As described in the specification (at page 7, lines 22-36), the three dimensional structure of a parent enzyme may be prepared by model building using the structure of a related protein. For example, the structure of a parent maltogenic alpha-amylase (the commercial product Novamyl ®) was model built on the structure disclosed in Appendix 1, and the structure of other maltogenic alpha-amylase can be model built analogously. The specification also describes (at page 7, lines 22-36) an example of how a model building procedure could be carried out:

A model structure of a maltogenic alpha-amylase can be built using the Homology program or a comparable program, e.g., Modeller (both from Molecular Simulations, Inc., San Diego, CA). The principle is to align the sequence of the maltogenic alpha-amylase with the known structure with that of the maltogenic alpha-amylase for which a model structure is to be constructed. The structurally conserved regions can then be built on the basis of consensus sequences. In areas lacking homology, loop structures can be inserted, or sequences can be deleted with subsequent bonding of the necessary residues using, e.g., the program Homology. Subsequent relaxing and optimization of the structure should be done using either Homology or another molecular simulation program, e.g., CHARMM from Molecular Simulations.

Moreover, the ability to prepare reliable three-dimensional models of other related proteins, once the critical step was provided by the inventors (i.e., the first structure of a maltogenic alpha-amylase), is further illustrated by the following references (copies are submitted herewith as Exhibits 1-5):

- 1) Greer J. 1990. Comparative Modeling Methods: Application to the Family of the

mammalian Serine Proteases. Publishers: Wiley-Liss, Inc. Proteins: Structure, Function, and Genetics 7:317-334.

- 2) Greer J. 1985. Protein Structure and Function by Comparative Model Building. Annals of The New York Academy of Sciences, vol. 439: 4463.
- 3) Greer J. 1981. Comparative Model-building of the Mammalian Serine Proteases. J Mol Biol 153:1027-1042.
- 4) Thirup and Jones. 1986. Using known substructures in protein model building and crystallography. J EMBO 5(4):819-822.
- 5) Blundell T.L. *et al.* 1987. Knowledge-based prediction of protein structures and the design of novel molecules. Nature 326(26):347-352.

Once the three dimensional model of the parent enzyme is prepared, the artisan is then able to select amino acids in the structure which can be modified so as to improve the activity of the enzyme. In this regard, the claims also recite the steps of:

- b) identifying in said three-dimensional structure of the parent alpha-amylase an amino acid residue which (i) is within 15 Å of one of said three amino acid residues and (ii) is involved in electrostatic or hydrophobic interactions with an active site residue; and
- c) constructing the variant by substituting the amino acid residue identified in said b) with another amino acid residue which changes the electrostatic and/or hydrophobic surroundings of said active site residue, and which can be accommodated in the structure.

The specification provides both general and specific guidance as to how to carryout these step, including guidance as to which specific amino acid residues to target and what changes to make. Foremost, the claims themselves recite that the amino acids (to target) should be within 15 Å or 10 Å of the active site residues. The ability to identify the amino acid residues that are within 15 Å or 10 Å of one of the recited active site residues is a task that is well within the practice of the skilled artisan, using, e.g., computer programs available at the time of the invention. For example, the specification describes how an artisan can identify specific amino acids within a site of interest using the computer program INSIGHT (BIOSYM Technologies) by distance from the site. See, e.g., the specification at page 19, lines 9-19. Although this section of the specification addresses a zone of interest of 10 Å around calcium ions in the three-dimensional structure, such computer programs could also be used to identify other amino acid zones of interest, including, amino acid residues within 15 or 10 Å of the recited active site residues.

The active site residues for SEQ ID NO:2 are identified on page 5 of the specification as "a cluster of three amino acid residues, D329, D228 and E256, spatially arranged at the bottom of a

cleft in the surface" of the enzyme in Domain A. The claims are directed to constructing variants of parent maltogenic alpha amylases which are highly related to SEQ ID NO:2. Accordingly, the claims recite that the parent maltogenic alpha-amylase "has an active site which comprises a cluster of three amino acid residues corresponding to positions D329, D228 and E256 in SEQ ID NO:2." As previously discussed, by virtue of their identity (i.e., 70% identity) to SEQ ID NO:2, the determination of what is a corresponding amino acid is a task that is well within the skill of the artisan and can be readily determined between highly related proteins, e.g., by aligning the parent maltogenic-alpha amylase to the reference sequence. See the specification at page 20, lines 10-21.

In addition to the criteria that an amino acid residue is within 15 Å or 10 Å of one of the three recited amino acid residues, the claims also recites that the amino acid residue "is involved in electrostatic or hydrophobic interactions with an active site residue." In this regard, an artisan can select amino acid residues having a characteristic, such as, based on the charge of amino acids or based on its hydrophobicity, which are known to effect the electrostatic or hydrophobic interactions (and consequently the pH dependent profile of the enzyme). The specification also provides examples of considerations an artisan would undertake when identifying appropriate target amino acid residues which are involved in electrostatic or hydrophobic interactions with an active site residue and making changes to such residues (e.g., to change the pH dependent profile). For example, the specification discloses that:

To obtain a higher activity at a higher pH, negatively charged residues are placed near a hydrogen donor acid, whereas positively charged residues placed near a nucleophilic acid will result in higher activity at low pH. Also, a decrease in the pKa can be obtained by reducing the accessibility of water or increasing hydrophobicity of the environment.

See the specification at page 9, line 19-23.

The claims also recite an important underlying general consideration when carrying out steps b and c, namely, that the changes made to the parent maltogenic alpha-amylase should "accommodate" the structure. Although this language is recited in the claim language itself, even without it, an artisan would clearly understand that the amino acid changes must not sterically hinder or otherwise disrupt the structure of the enzyme such that the enzyme is not able to function, e.g., to bind its substrate.

The specification further provides detailed examples of residues within 15 or 10 Å which can be modified to alter the pH dependent activity profile of the enzyme, including, specific

substitutions for such residues. See the specification at page 10, lines 3 to 24. Similar modifications can also be introduced in equivalent positions of other parent maltogenic alpha-amylases.

Thus, it is respectfully submitted that the scope of protection sought by the claims is commensurate in scope with the enablement provided in the specification. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claim 5 under 35 U.S.C. 112, Enablement

Claim 5 is further rejected under 35 U.S.C. 112, first paragraph, as allegedly lacking enablement, for the reason that the specification does not enable an artisan to model the parent alpha-amylase on the three-dimensional structure of amino acid residues 1-686 of SEQ ID NO:2 depicted in the Appendix to produce a three-dimensional structure of the parent alpha-amylase. The Examiner states that although reference is made to modeling programs in the art, this is not specific guidance for modeling a parent maltogenic alpha-amylase. This rejection is respectfully traversed.

As discussed above, the high degree of structural relatedness between sequences that are at least 70% identical to amino acid residues 1-686 of SEQ ID NO:2 means that one of ordinary skill in the art, based on the X-ray crystallographic structure of Appendix 1, would be able to prepare a three-dimensional model of the parent maltogenic alpha amylase and identify target sites for mutation in any member of the recited family. Although Appendix 1 provides the atomic co-ordinates (and the three-dimensional structure) of a specific maltogenic alpha-amylase (i.e., the maltogenic alpha amylase having amino acids 1-686 of SEQ ID NO:2), three-dimensional models of other homologous enzymes can be readily prepared by the skilled artisan, as recited in the claims, by "modeling the parent alpha-amylase on the three-dimensional structure of amino acid residues 1-686 depicted in the Appendix." That is, Appendix 1 serves as a suitable template for other homologous enzymes.

Accompanying this amendment as Exhibits 1-5 are numerous scientific articles which further support that the skilled artisan is able to prepare a model build using the three-dimensional structure of a sufficiently homologous structure. Applicants have not provided a detailed description of a step-by-step process for running modeling computer programs, however, such information is know in the art, and therefore need not be disclosed.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 5 and 35 under 35 U.S.C. 112, Written Description

Claims 5 and 35 are rejected under 35 U.S.C. 112, first paragraph, for allegedly lacking adequate written description support. The Examiner alleges that claimed invention is not sufficiently described because there is no disclosure of all three-dimensional (i.e., coordinates) crystal structure (i.e., parent maltogenic alpha-amylases having at 70% identity to amino acids 1-686 of SEQ ID NO:2) and because there is no disclosure of the active site, specific sequence, atomic coordinates and active site locations of parent alpha-amylase other than SEQ ID NO:2. This rejection is respectfully traversed, as applied to the new claims.

As discussed in the context of the enablement rejection, the high degree of structural relatedness between sequences that are at least 70% identical to amino acid residues 1-686 of SEQ ID NO:2, means that one of ordinary skill in the art, based on the X-ray crystallographic structure of Appendix 1, would be able to prepare a three-dimensional model of the parent maltogenic alpha-amylase and identify target sites for mutation in any member of the recited family. In this regard, it is not necessary for the Applicants to specifically disclose the three-dimensional coordinates of parent maltogenic alpha-amylases having at least 70% identity to amino acids 1-686 of SEQ ID NO:2 because such information is obtained by modeling the parent alpha-amylase on the three-dimensional structure of amino acid residues 1-686 depicted in the Appendix. That is, although Appendix 1 provides the atomic coordinates and the three-dimensional structure of a specific maltogenic alpha-amylase, Appendix 1 is also a template and which can be used to prepare three-dimensional models of other enzymes which sufficient degree of identity. See, e.g., the articles provides in Exhibits 1-5 , which discuss the ability of the artisan to build models from sufficiently homologous structures, and thus obtain the information the Examiner alleges is missing from the specification.

With respect to the sequence information alleged to be absent from the disclosure, such information is clearly not required as the sequence of a parent maltogenic alpha-amylase can be obtained using techniques which are well-known in the art for determining the amino acid sequence or nucleic acid sequence of a protein.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. The Rejection of Claims 5 and 35-40 under 35 U.S.C. 112, Indefiniteness

Claims 5 and 35-40 are rejected under 35 U.S.C. 112, as vague and indefinite for a number of reasons. The rejections are addressed as applied to the new claims 41-57.

The Examiner contends that the claims are vague and indefinite because it is allegedly unclear what is meant by the term "accommodated". The Examiner states that Applicants can resolve this issue by particularly point out what the term "accommodated in the structure" refers to.

The term "accommodated" is used in its plain and ordinary meaning in the art to refer to an acceptable fit, and the phrase "accommodated in the structure" accordingly refers to the amino acid changes made to the parent enzyme, and whether such changes fit in the structure of the enzyme. A clear example of the meaning of the term would be that as a result of the amino acid change, the structure is not sterically disrupted such that the enzyme is no longer able to bind the substrate. (As is well-known in the art, such steric analysis could be readily performed using, e.g., substrate analogs and visualizing the interaction, i.e., the ability of the modified enzyme to bind its substrate.)

Applicants also refer the Examiner to Exhibit 6, "Steric Constraints In Model Proteins," arXiv:cond-mat/971277 v1 (Dec. 23, 1997), at page 3, last paragraph, to support that the term "accommodated" is, in fact, commonly used in the art and would be understood when referencing acceptable amino acid changes made in proteins.

Claim 5 is also rejected based on the use of the phrase "optionally repeating." The Examiner states it is unclear how many times the steps can be repeated and the criteria by which the option is set forth.

As amended, the "optional" repeating step is now recited in a dependent claim. The recitation of the number of times to repeat the steps, however, is clearly not necessary to impart an understanding of what is intended by this phrase. Rather, this phrase simply conveys that an artisan may repeat the steps to further improve the enzyme.

Moreover, the criteria by which the option is set forth is appropriately left to the discretion of the artisan practicing the claimed invention and need not be recited for imparting an understanding of when to exercise the option. For example, if the artisan is satisfied with the improvement resulting from carrying out the steps only once, the artisan may not want to further improve the enzyme by repeating the steps. If the artisan wants to further improve the enzyme for any number of reasons (e.g., further commercial improvements or even scientific curiosity), the artisan may want to repeat the recited steps.

This same reasoning also traverses the Examiner's assertions that the phrases "optionally substituting" and "optionally repeating step a)-g) recursively" are also vague and indefinite.

The Examiner further contends that the claims are indefinite because the body of the claim does not correspond to the preamble language, in that the basic step must be recited in a positive, active fashion. The new claims now employ in the body (step c) the phrase "constructing the variant", which is the same language used in the preamble. Applicants respectfully submit that, as applied to new claims, this rejection is moot.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

V. Objection to the Disclosure

The specification is objected to for incorrectly referencing SEQ ID NO:1 as the amino acid sequence instead of SEQ ID NO:2. SEQ ID NO:1 includes both a nucleic acid sequence and an amino acid sequence. However, in order to expedite prosecution, the specification has been made amended to reference the amino acid of SEQ ID NO:2.

For the foregoing reasons, Applicants submit that the claims overcome this objection.

VI. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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